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ARTICLE

Phenolic compounds in different parts of young *Annona muricata* cultivated in Korea and their antioxidant activity

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Abstract The antioxidant activities of 80% methanol and distilled water extracts of different parts (roots, twigs, and leaves) of young *Annona muricata* were estimated based on their total phenol and flavonoid content as well as in vitro 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activities, nitrite scavenging activity, Fe^{2+}-chelating activity, ferric reducing antioxidant power (FRAP), and phenolic compound assays. The 80% methanol extracts showed a higher antioxidant effect than that of the water extracts. The 80% methanol root and leaf extracts showed higher total phenol (839.69 mg CAE/g) and flavonoid (168.52 mg RE/g) contents than those shown by the other extracts. In addition, the 80% methanol root extracts showed high DPPH (EC_{50} = 0.18 mg/mL) and ABTS (EC_{50} = 0.55 mg/mL) radical as well as nitrite (EC_{50} = 0.21 mg/mL) scavenging activities. The metal-chelating effect of the 80% methanol twig extract was the highest, but there were no significant differences among the 80% methanol extracts of the different parts. FRAP values of all extracts increased in a concentration-dependent manner, except for those of the distilled water leaf extract, while the 80% methanol root extracts showed the highest value. In addition, there was a strong positive correlation between the antioxidant activity and total phenol content ($P < 0.01$). *A. muricata* extracts were rich in various phytochemicals including rutin, epicatechin, ferulic acid, and *p*-coumaric acid. These findings indicate that *A. muricata* is a potentially useful source of substances with antioxidant effects.

Keywords *Annona muricata* · Antioxidant activity · Correlation · Graviola · Phenolic compounds

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Introduction

Reactive oxygen species (ROS) generated during metabolic processes in the human body are eliminated by enzymatic and non-enzymatic antioxidant systems that exist in the body. However, antioxidant system abnormalities, or excess generation of ROS due to various physical and chemical factors, induce oxidative stress, which causes tissue damage and gene mutation, resulting in various chronic diseases, such as diabetes, Alzheimer's disease, and aging [1]. There is increasing interest in preventing these and other metabolic syndromes. Thus, research on the development of functional materials from edible natural resources with antioxidant effects and few side effects is actively in progress [2, 3].

Annona muricata is a tree that belongs to the Annonaceae family, and its fruits are used as ingredients in foods and drinks [4]. In Korea, following the introduction of *A. muricata* in the Siheung region of Gyeonggi in 2014, the fruit is increasingly cultivated owing to the identification of its bioactive substances and biological effects [5, 6]. *A. muricata* contains 212 different bioactive substances including acetogenins, alkaloids, and phenols. Furthermore,

it is known to be effective in relieving cough associated with mild skin diseases and asthma and has been used as a traditional medicine in Central and South America, West Africa, and Southeast Asia [7]. The phenolic compounds in *A. muricata* fruit pulp, as well as the antioxidant, antidiabetic, analgesic, and anti-inflammatory effects of aqueous and ethanolic leaf extracts were recently analyzed [8–11]. Further, according to recent studies, *A. muricata* is effective in wound healing, has antibacterial activity, reduces high blood pressure, and, in particular, induces cancer cell cytotoxicity, thereby exerting anticancer effects [12–15].

Although *A. muricata* is a useful plant resource with various bioactivities, its antioxidant activity and active components are not well studied in Korea. Further, most research studies have focused on the leaves and fruit, but root- and twig-based substances are yet to be discovered. Therefore, in the present study, we evaluated the extracts of different plant parts of young *A. muricata* cultivated in Korea to verify their antioxidant activities. In addition, we separated the active components using high-performance liquid chromatography (HPLC) to identify substances for possible development as new functional products.

Materials and methods

Experimental materials and reagents

The *A. muricata* used in this study was cultivated on farms in Gwacheon, Gyeonggi. Samples were sliced into appropriate sizes, freeze-dried (FD-5512, Ilshin Lab. Co. Ltd., Gyeonggi, Korea), pulverized, filtered through a 40-mesh filter, and stored at −70 °C. Folin–Ciocalteu's reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-*p*,*p*′-disulfonic acid monosodium salt (ferrozine), 2,4,6 tris(2-pyridyl) 1,3,5-triazine (TPTZ), Griess reagent, ethylenediamine tetraacetic acid, chlorogenic acid, rutin, gallic acid, epigallocatechin, catechin, caffeic acid, epicatechin, epigallocatechin gallate, *p*-coumaric acid, gallocatechin gallate, ferulic acid, epicatechin gallate, catechin gallate, naringin, and quercetin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other reagents used were of analytical grade.

Preparation of extracts

To prepare extracts, 20 g of *A. muricata* powder was extracted with 200 mL of distilled water or 80% (v/v) methanol in a shaking water bath (BS-21, Jeio Tech Co., Gyeonggi, Korea) for 24 h, followed by centrifugation (Avanti J-26 XPI, Beckman Coulter, Fullerton, CA, USA) at 11,325×*g* for 30 min. The supernatant was filtered through filter paper (Whatman No. 1, Maidstone, England) and then concentrated using a rotary vacuum evaporator (R-210, Buchi, Flawil, Switzerland), followed by freeze-drying to remove the solvent.

Determination of total polyphenol content

The method of Folin–Ciocalteu [16] was used to analyze the total polyphenol content by treating 50 μL samples of different concentrations with 25 μL 2 N Folin–Ciocalteu's reagent and 150 μL 20% sodium bicarbonate (Na_2CO_3). This was followed by incubation at 20 °C for 15 min, and then, the optical density was measured using an enzyme-linked immunosorbent assay (ELISA) plate reader (VersaMax Molecular Devices, Sunnyvale, CA, USA) at 725 nm. The total polyphenol content was calculated from a standard calibration curve constructed from 0 to 200 μg/mL chlorogenic acid and was expressed as milligram of chlorogenic acid equivalents (CAE) per gram of extract.

Determination of total flavonoid content

The method of Zhishen et al. [17] was used to determine the total flavonoid content by reacting 20 μL of the prepared extracts at different concentrations with 200 μL diethylene glycol and 20 μL 1 N sodium hydroxide (NaOH) at 40 °C for 1 h. This was followed by measuring absorbance at 420 nm using an ELISA plate reader. Rutin (0–1000 μg/mL) was used as a standard to construct a calibration curve, and the results of the total flavonoid content were calculated as milligram of rutin equivalents (RE) per gram of extract.

DPPH radical scavenging activity assay

A modification of the method of Blois [18] was used to measure the DPPH radical scavenging activity of the extracts. Different concentrations of the prepared extracts (100 μL) were mixed with 900 μL 0.1 mM DPPH solution and then were reacted in the dark for 30 min. This was followed by optical density measurement at 517 nm using an ELISA plate reader. The DPPH radical scavenging activity (%) is shown as the optical density ratio of the sample- and non-treated groups, which was used to calculate the effective concentration (EC_{50}) value, representing the sample concentration with 50% radical scavenging activity.

ABTS radical scavenging activity assay

A partial modification of the method of Pellegrini et al. [19] was used to measure the ABTS radical scavenging

activity. Briefly, 5 mL 7 mM ABTS and 88 μL 140 mM potassium persulfate were mixed and kept in the dark at 20 °C for 15 h to form radicals. Then, the solution was diluted to obtain an optical density value of 0.7 ± 0.02 at 734 nm. One milliliter of this diluted ABTS solution was added to 50 μL extract, which was kept in the dark for 10 min, and the optical density was subsequently measured at 734 nm. The ABTS radical scavenging activity (%) was calculated as the difference in optical densities between the sample-treated and non-treated groups to calculate the EC_{50} value.

Nitrite scavenging activity assay

The method of Kato et al. [20] was used to measure the nitrite scavenging activity of the extracts. Different concentrations of the prepared extracts (40 μL) were mixed with 20 μL of 1 mM sodium nitrite ($NaNO_2$), and then, 140 μL 0.1 N HCl was added to adjust the pH of the reaction solution to 1.2. The reaction was incubated at 37 °C for 1 h. To the reaction solution, 1 mL 5% (v/v) acetic acid and 80 μL Griess reagent were added and reacted at 20 °C for 15 min. Then, the optical density was measured at 520 nm. The nitrite scavenging activity (%) was expressed as the percentage difference in optical density between the sample-treated and non-treated groups to calculate the EC_{50}.

Metal (Fe^{2+})-chelating activity assay

The method of Dinis et al. [21] was modified and used to measure the Fe^{2+}-chelating activity. Briefly, different concentrations of the prepared extracts (1 mL) were treated with 25 μL 2 mM $FeCl_2$ and 5 mM ferrozine at 20 °C for 10 min, and the optical density was then measured at 562 nm. The Fe^{2+}-chelating activity was expressed as the percentage difference in optical density between the sample-treated and non-treated groups to calculate the EC_{50} value.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed using the method of Benzie and Stain [22] to measure the antioxidant activity. Briefly, 30 mM acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM $FeCl_3 \cdot 6H_2O$ were mixed at a 10:1:1 ratio to produce a reaction solution (700 μL), which was used to treat different concentrations of the formulated extracts (100 μL) for 30 min in the dark. The optical density was measured using an ELISA plate reader at 590 nm. The FRAP values were calculated as μM trolox equivalents (TEs) using calibration curves constructed with trolox as the standard.

Analysis of phenolic compounds using HPLC

The extract was dissolved in 50% methanol, filtered using a syringe filter (0.45 μm). The solution (10 μL) was injected into an HPLC system (Agilent 1200 series, Agilent Technologies, Palo Alto, CA., USA.) that was equipped with a Gemini C_{18} column (3-μm, 150 × 4.6 mm, Phenomenex, Casalecchio di Reno, Bologna, Italy). The detector used for the analysis was a diode-array detector (DAD) at 220 nm, and 0.2 M phosphoric acid (solvent A) and acetonitrile (solvent B) were used as the mobile phases. The conditions for running the mobile phase were 0–20 min B: 0 → 15%, 20–40 min B: 15 → 35%, and 40–50 min B: 35 → 90% at a flow rate of 1.0 mL/min.

Statistical analysis

The results are presented as the means ± standard deviations of at least three independent determinations, and the SPSS program (ver. 10.0, SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. One-way analysis of variance (ANOVA) was used to evaluate the differences among the various extracts, and Duncan's multiple range tests were conducted as the post hoc analysis. Differences were considered significant at $P < 0.05$. The correlation between the antioxidant substance and activity at $P < 0.01$ and $P < 0.05$ levels was analyzed by calculating Pearson's correlation coefficient to obtain the coefficient of determination (r^2) values.

Results and discussion

Yield and total polyphenol and flavonoid content

The yield, total polyphenol, and flavonoid contents of the 80% methanol and distilled water extracts of young *A. muricata* roots, twigs, and leaves are listed in Table 1. The yield of the 80% methanol extract was the highest for the roots (11.00%), compared to that for the twigs (5.35%) and leaves (10.30%), and the yield of the water extract was the highest for the leaves (15.50%). The yield was higher for the water extracts than it was for the 80% methanol extracts for the twigs and leaves, but not for the roots. George et al. [23] reported that the yield of the methanol extract of Indian *A. muricata* leaves was 7.12% and that of the distilled water extract was 15.00%, a similar trend to the results of our study. El-Chaghaby et al. [24] extracted Egyptian sugar apple leaves with various solvents and obtained yields in the order of 50% acetone (10.36%) > distilled water (10.34%) > 50% ethanol (6.97%) > 80% methanol (5.76%), which shows a similar

Table 1 Yield, total phenolic content, and total flavonoid content of different parts of *Annona muricata*

	Yield[1)] (%)	TPC[4)] (mg CAE/g)	TFC (mg RE/g)
80% methanol			
Roots	11.00	839.69 ± 0.72[2),a,3)]	24.49 ± 1.59[bc]
Twigs	5.35	705.94 ± 11.76[c]	20.19 ± 1.63[c]
Leaves	10.30	763.36 ± 11.65[b]	168.52 ± 2.35[a]
Distilled water			
Roots	10.60	106.78 ± 0.66[e]	3.85 ± 0.29[d]
Twigs	6.15	102.03 ± 0.66[f]	3.05 ± 0.21[e]
Leaves	15.50	155.19 ± 0.14[d]	25.79 ± 0.12[b]

[1)] Extraction yield was calculated as % yield = (weight of sample extract/initial weight of sample) × 100

[2)] Values are mean ± SD in triplicate

[3)] Values in the same column with different letters are significantly different at $P < 0.05$

[4)] *TPC* total phenolic content, *TFC* total flavonoid content, *CAE* chlorogenic acid equivalents, *RE* rutin equivalents

trend to the results of our study, although the yield was higher with *A. muricata* than with sugar apple.

Polyphenols are secondary metabolites generated through photosynthesis in the plant. The hydroxyl groups (–OH) in polyphenols, which characteristically bind with proteins and various compounds, are known to exhibit bioactivities, such as antioxidant, anticancer, and antiobesity effects [25, 26]. Flavonoids are a type of polyphenol with a basic flavone structure, and they occur in high amounts in plant stems and fruits. Furthermore, they have been reported to have various functionalities, such as antioxidant and anti-inflammatory effects [27]. The results of the total polyphenol and flavonoid content of the different parts of *A. muricata* using different extraction solvents are listed in Table 1.

The samples extracted with 80% methanol show the highest and lowest total polyphenol content in the roots and twigs at 839.69 ± 0.72 and 705.94 ± 11.76 mg CAE/g, respectively. The distilled water leaf extracts contained the most polyphenol at 155.19 ± 0.14 mg CAE/g, while the twigs contained the least at 102.03 ± 0.66 mg CAE/g. When the same parts were compared based on extraction solvent, the 80% methanol extract of the roots, twigs, and leaves showed a 7.8-, 6.9-, and 4.9-fold higher content, respectively, than that of the corresponding distilled water extracts. El-Chaghaby et al. [24] compared the total phenol content in sugar apple leaf extracts and found 353 ± 20.88 and 210 ± 59.03 mg GAE/g in the methanol and water extracts, respectively. This trend was similar to that observed in our study, with a higher content in the methanol than in the water extract, although there was a difference in the amounts extracted. Further, the results also show a large difference between the total phenol content of the Indian *A. muricata* methanol leaf (36.2 μg GAE/g) and water (19.1 μg GAE/g) extracts [23]. This variation in content could be due to the difference in the climate, such as temperature, humidity, and wind, in the areas where *A. muricata* was grown and cultivated.

The total flavonoid content of the 80% methanol and distilled water extracts of the leaves was 168.52 ± 2.35 and 25.79 ± 0.12 mg RE/g, respectively, which was higher than that of roots and twigs, while the content of the water extract of the twigs was the lowest of the six extracts at 3.05 ± 0.21 mg RE/g. When the same plant parts were compared based on the extraction solvent, the 80% methanol extracts showed an approximately 6-fold higher flavonoid content with all parts than the distilled water extracts did. Mariod et al. [28] measured the total flavonoid content of methanol extracts of sugar apple leaves and roots, and the results were reported to be 222.6 and 106.6 mg RE/g, respectively, indicating higher flavonoid content than that of *A. muricata*. However, both plant species had higher flavonoid levels in the leaves than in the roots. Most studies have reported high polyphenol and flavonoid content in leaves, which could be due to the accumulation of polyphenols such as catechin, generated during photosynthesis in the leaves [29].

Antioxidant activities

Plants contain various antioxidant substances that are produced by different mechanisms. Hence, more than one method needs to be used to verify accurately the antioxidant activity [30]. Therefore, in this study, we used five different antioxidant measurement methods and calculated the EC_{50} in four of them, with the exception of the FRAP assay, and the results are listed in Table 2.

For the DPPH radical scavenging activity, the EC_{50} values were the highest for the roots and leaves extracted with 80% methanol at 0.18 ± 0.00 mg/mL for both, while the magnitude of the activity of the distilled water extracts was in the order of leaves > roots > twigs. A previous study showed that the distilled water extract of *A. muricata* leaves had a higher DPPH radical scavenging activity (IC_{50} = 0.9077 mg/mL) than that of ethanol extract (IC_{50} = 2.0456 mg/mL) [10], which is similar to that of the distilled water extract of *A. muricata* leaves obtained in our study (EC_{50} = 0.81 ± 0.02 mg/mL). However, the results of our study differed from those of Mariod et al. [28], who reported IC_{50} values of methanol extracts of both sugar apple leaves and roots to be 7.81 ± 0.1 μg/mL. Park et al. [31] measured DPPH radical scavenging activities of extracts and fractions of *Ligularia fischeri* Turcz leaves and found that the ethanol extracts had higher activities than the water extracts did.

Table 2 Half-maximal effective concentration (EC_{50}) values of different plant parts of *Annona muricata*

	EC_{50}[1] (mg/mL)			
	DPPH[2]	ABTS	Nitrite	Fe
80% methanol				
Roots	0.18 ± 0.00[3),e,4)]	0.55 ± 0.01[e]	0.21 ± 0.01[d]	0.39 ± 0.01[c]
Twigs	0.24 ± 0.01[d]	0.89 ± 0.03[d]	0.37 ± 0.00[d]	0.33 ± 0.00[c]
Leaves	0.18 ± 0.00[e]	0.84 ± 0.02[d]	0.32 ± 0.01[d]	0.42 ± 0.01[c]
Distilled water				
Roots	1.76 ± 0.05[b]	5.20 ± 0.06[a]	4.91 ± 0.20[a]	13.09 ± 0.39[b]
Twigs	2.15 ± 0.04[a]	4.53 ± 0.10[b]	4.58 ± 0.10[b]	16.43 ± 0.88[a]
Leaves	0.81 ± 0.02[c]	2.04 ± 0.03[c]	1.84 ± 0.01[c]	0.57 ± 0.01[c]

[1)] EC_{50} values are expressed as the effective concentration required to obtain a 50% antioxidant effect

[2)] *DPPH* DPPH radical scavenging activity, *ABTS* ABTS radical scavenging activity, *nitrite* nitrite scavenging activity, *Fe* Fe^{2+}-chelating activity

[3)] Values are mean ± SD in triplicate

[4)] Values in the same column with different letters are significantly different at $P < 0.05$

The ABTS radical scavenging activity results showed that the EC_{50} value of the 80% methanol root extract was the highest at 0.55 ± 0.01 mg/mL, followed by the leaves and twigs in that order. The distilled water extract of the leaves showed an EC_{50} of 2.04 ± 0.03 mg/mL, which was the highest activity, while the roots showed the lowest activity at 5.20 ± 0.06 mg/mL. Ajboye et al. [32] reported that 200 μg/mL leaf water extract of custard apple of the genus *Annona* showed ABTS radical scavenging activity that was >60% of the value that we obtained. Further, Baskar et al. [33] measured the ABTS radical scavenging activity of the ethanol leaf extracts of Annonaceae plants cultivated in India and reported the IC_{50} of *A. muricata* to be 305 μg/mL, which differed from the results of our study.

The EC_{50} value for the nitrite scavenging activity was highest for the 80% methanol extract of the roots at 0.21 ± 0.01 mg/mL, but this was not significantly different from the values of the twigs or roots ($P > 0.05$). For the distilled water extracts, the leaves and roots showed the highest and lowest activities (EC_{50} = 1.84 and 4.91 mg/mL), respectively, and there was a significant difference among the parts ($P < 0.05$). Baskar et al. [33] reported that the leaf ethanol extracts of Indian *A. muricata* showed an IC_{50} value of 350 μg/mL, which is lower than that of the 80% methanol extract and higher than that of the distilled water extract of the leaves measured in our study. Further, the nitrite scavenging activity of same solvent extracts was higher from the leaves of *Cudrania tricuspidata* than it was from the stems [34], which corresponds to the results of our study.

The metal ion (Fe^{2+})-chelating activity was the highest in the 80% methanol extract of the twigs at 0.33 ± 0.00 mg/mL, but this value did not differ significantly from that of the roots and leaves ($P > 0.05$). Furthermore, the magnitude of the metal-chelating activity of the distilled water extracts was in the following order: leaves > roots > twigs, and the difference was significant ($P < 0.05$). These results differed from those of the study by Öztürk et al. [35], who reported the highest metal-chelating activity in the stems when methanol extracts of rhubarb roots and stems were analyzed. In addition, the study by Okoko and Ere [36] reported a 40% metal-chelating effect of the methanol extract of papaw tree leaves at 2 mg/mL concentration.

The FRAP assay, which is fast and convenient, is based on the principle that the ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to ferrous tripyridyltriazine (Fe^{2+}-TPTZ) in an acidic environment by a reducing agent [22]. The FRAP values of different concentrations of the 80% methanol and distilled water extracts of *A. muricata* roots, twigs, and leaves were measured, and the results are shown in Fig. 1. Overall, the 80% methanol extracts showed higher values than those of the distilled water extracts, and they exhibited concentration-dependent responses, except at 1000 μg/mL. The 80% methanol root and distilled water leaf extracts showed the highest FRAP activities (1201.12 and 654.46 μM TE, respectively). George et al. [23] analyzed the FRAP activity of *A. muricata* leaf extracts and reported that the effects of methanol extracts were higher than those of distilled water extracts, which corresponds to the results of our study. The results of our study are also in agreement with those of Michel et al. [37], who reported the magnitude of the FRAP activity of organic solvent extracts of sea buckthorn parts to be in the following order: roots > leaves > stems.

These results demonstrate that the antioxidant effect of the 80% methanol extracts was higher than that of the distilled water extracts for all parts of *A. muricata*. In

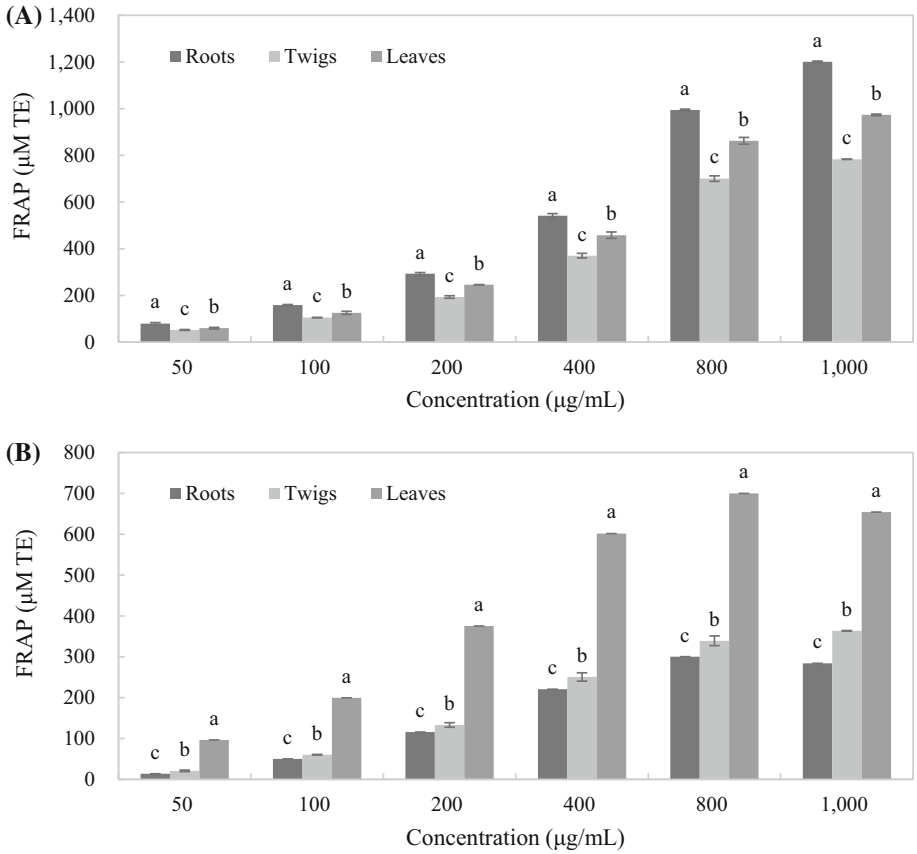

Fig. 1 Ferric reducing antioxidant power (FRAP) values of (**A**) 80% methanol and (**B**) distilled water extracts from different parts of *Annona muricata*. Each value represents the mean ± SD of triplicate measurements. One-way analysis of variance (ANOVA) was used to evaluate the differences among the various extracts, and Duncan's multiple range tests were conducted as the post hoc analysis. Values with different letters in the same concentration are significantly different at $P < 0.05$. TE, trolox equivalent

particular, the 80% methanol extract of the roots exhibited the highest effects in all of the antioxidant evaluation methods, excluding metal-chelating activity. The distilled water extracts of the leaves showed the lowest (2-fold) to the highest (>22-fold) antioxidant activity of all of the parts. These results could be due to the different contents of antioxidant substances in various plant parts, and the extraction efficiency in different solvents.

Phenolic compounds

To identify substances with antioxidant activity in *A. muricata* extracts according to the extraction solvent, HPLC was used to analyze the phenolic compound composition, and the results are listed in Table 3. From 15 potential substances, 14 were detected (quercetin was not detected), and the main phenolic compounds in the 80% methanol extract of the roots were ferulic acid, epicatechin, and caffeic acid, within the range of 1585.90–1893.30 mg/100 g. The phenolic compounds of the methanol extract of the twigs were in the following decreasing order: epicatechin > *p*-coumaric acid > catechin, and the contents of the methanol extract of the leaves were in the order of rutin > epicatechin > catechin gallate. In particular, the rutin content in the methanol extract of *A. muricata* leaves was 4758.90 mg/100 g, which was the highest.

The main phenolic compounds in distilled water extracts of the roots were *p*-coumaric acid, ferulic acid, and epicatechin gallate, which were detected within the range of 93.53–161.08 mg/100 g, approximately one-tenth of those of the methanol extracts. In the water extract of the twigs, only five phenolic compounds, including epicatechin (86.91 mg/100 g) were detected, and compounds in the water extract of the leaves were detected in the order of rutin > chlorogenic acid > naringin, indicating a marked difference in the phenolic compound content based on the solvent and plant part. In the study by George et al. [23], phenolic compound analysis of the methanol extracts of Indian *A. muricata* leaves detected quercetin, catechin, gallocatechin, and epigallocatechin in the water extracts, in contrast to our study. Such a difference in the type and the content of phenolic compounds could be due to environmental factors (i.e., the cultivation region) and different eluted amounts of phenolic compounds (i.e., the extraction method).

According to the analysis of the ethanol extracts of Egyptian *A. muricata* leaves by Nawwar et al. [38], phenolic compounds such as catechin, epicatechin, chlorogenic acid, and rutin were detected and were reported to be the main substances mediating the antioxidant activity of the plant. Further, caffeic acid and ferulic acid, which were detected in all of the extracts in this study, exist abundantly

Table 3 Phenolic compounds (mg/100 g) of different parts of *Annona muricata*

	Compounds	80% methanol			Distilled water		
		Roots	Twigs	Leaves	Roots	Twigs	Leaves
1	Gallic acid	174.03 ± 19.56[1),a,2)]	–[3)]	–	–	–	–
2	Epigallocatechin	–	295.70 ± 20.34[a]	–	–	–	50.07 ± 1.08[b]
3	Catechin	803.13 ± 11.13[a]	686.13 ± 10.40[b]	439.33 ± 6.09[c]	38.17 ± 2.40[ef]	49.82 ± 3.11[e]	174.57 ± 2.27[d]
4	Chlorogenic acid	–	–	–	48.51 ± 0.97[b]	–	469.54 ± 6.25[a]
5	Caffeic acid	1585.90 ± 46.84[a]	466.90 ± 33.07[c]	860.10 ± 108.97[b]	34.30 ± 4.18[de]	42.78 ± 7.69 [cd]	28.96 ± 0.13[e]
6	Epicatechin	1774.53 ± 24.08[b]	1213.87 ± 55.47[c]	2075.17 ± 78.56[a]	78.06 ± 5.15[e]	86.91 ± 4.04[d]	88.68 ± 1.45[d]
7	Epigallocatechin gallate	463.93 ± 26.56[a]	–	–	65.79 ± 0.77[b]	–	–
8	*p*-Coumaric acid	659.70 ± 48.98[c]	784.40 ± 10.43[b]	967.23 ± 18.01[a]	161.08 ± 10.1[d]	–	28.54 ± 2.75[e]
9	Gallocatechin gallate	335.77 ± 2.73[a]	284.20 ± 10.00[b]	260.20 ± 9.18[c]	85.86 ± 4.48[d]	–	28.99 ± 1.15[e]
10	Ferulic acid	1893.30 ± 109.34[a]	361.00 ± 19.30[b]	319.53 ± 22.75[c]	128.14 ± 5.87[d]	25.92 ± 0.53[f]	93.83 ± 10.67[e]
11	Epicatechin gallate	399.39 ± 113.11[b]	–	904.83 ± 44.46[a]	93.53 ± 2.73[d]	32.58 ± 1.25[e]	189.24 ± 2.01[c]
12	Rutin	530.43 ± 177.20[c]	384.10 ± 8.32[d]	4758.90 ± 101.2[a]	–	–	608.77 ± 19.26[b]
13	Catechin gallate	299.33 ± 16.31[b]	246.37 ± 15.75[c]	1490.27 ± 44.83[a]	–	–	27.27 ± 0.47[d]
14	Naringin	339.83 ± 24.04[c]	–	1134.67 ± 30.24[a]	23.27 ± 1.23[d]	–	378.98 ± 32.63[b]
15	Quercetin	–	–	–	–	–	–

[1)] Values are mean ± SD in triplicate

[2)] Values in the same row with different letters are significantly different at $P < 0.05$

[3)] – Not detected

in plants that effectively eliminate radicals, such as ABTS, DPPH, and superoxide anion. In addition, caffeic and ferulic acid are known to exhibit bioactivities, such as reduction of lipid oxidation and protection of neurons [39, 40].

Therefore, the antioxidant activity of Korean *A. muricata* extracts is also likely to be related to these substances. Mariod et al. [28] detected chlorogenic acid (0.022 ± 0.01 mg/100 g) and ferulic acid (0.011 ± 0.21 mg/100 g) in the roots, and chlorogenic acid (0.351 ± 0.35 mg/100 g), ferulic acid (0.014 ± 0.13 mg/100 g), and *p*-coumaric acid (0.001 ± 0.01 mg/100 g) in the methanol extracts of sugar apple leaves. These values are lower than those of each of the phenolic compounds which are in *A. muricata*. Therefore, Korean *A. muricata* contains higher levels of phenolic compounds than the sugar apple does and thus could have enhanced antioxidant activity.

Correlation between antioxidant substance and activities

The phenolic compound contents of plants are known to be closely correlated with their radical scavenging and metal ion reducing activity [41]. Therefore, the correlation between antioxidant substance and activity was evaluated in extracts of different parts of the *A. muricata* plant according to extraction solvent, and the results are represented as the r^2 (Table 4). The total polyphenol content was highly correlated with all antioxidant activities ($P < 0.01$). In particular, the r^2 value in the FRAP analysis was the highest at 0.976 ($P < 0.01$).

The total flavonoid content was significantly correlated with DPPH radical scavenging activity and FRAP with r^2 values of 0.287 and 0.220 (both $P < 0.05$), respectively, but these r^2 values were low overall. Accordingly, the antioxidant activity of *A. muricata* is considered to be more affected by the total polyphenol content than it is by the total flavonoid content. The r^2 value of the DPPH radical scavenging activity was within the range of 0.839–0.893 ($P < 0.01$) and therefore was highly correlated with all antioxidant activities. Further, correlations with catechin, caffeic acid, epicatechin, ferulic acid, and rutin were all identified, and in particular, epicatechin showed the highest correlation ($r^2 = 0.820$, $P < 0.01$) of all of the phenolic compounds.

Tsai et al. [42] reported that the catechin and ferulic acid contents are highly correlated with DPPH and ABTS radical scavenging activities and that catechin has a close positive relationship with lipid peroxidation inhibition. Accordingly, not only epicatechin, but also catechin and

Table 4 Pearson's coefficients of determination (r^2) among TPC, TFC, and antioxidant activities of various extracts from *Annona muricata*

	TPC[1)]	TFC	DPPH	ABTS	Nitrite	Fe	FRAP	Catechin	Caffeic acid	Epicatechin	Ferulic acid	Rutin
TPC	1	0.267*[2)]	0.908**	0.893**	0.958**	0.605**	0.976**	0.882**	0.771**	0.931**	0.460**	0.250*
TFC		1	0.287*	0.171	0.186	0.163	0.220*	0.047	0.126	0.483**	−0.001	0.996**
DPPH			1	0.876**	0.859**	0.839**	0.893**	0.835**	0.584**	0.820**	0.317*	0.266*
ABTS				1	0.960**	0.757**	0.811**	0.895**	0.837**	0.790**	0.629**	0.157
Nitrite					1	0.627**	0.899**	0.914**	0.876**	0.872**	0.627**	0.171
Fe						1	0.576**	0.664**	0.366**	0.483**	0.226*	0.148
FRAP							1	0.882**	0.658**	0.872**	0.371*	0.203
Catechin								1	0.723**	0.679**	0.593**	0.038
Caffeic acid									1	0.746**	0.837**	0.118
Epicatechin										1	0.366**	0.464**
Ferulic acid											1	−0.001
Rutin												1

[1)] *TPC* total phenolic content, *TFC* total flavonoid content, *DPPH DPPH* radical scavenging activity, *ABTS* ABTS radical scavenging activity, *nitrite* nitrite scavenging activity, *Fe* Fe^{2+}-chelating activity

[2)] Significant at * $P < 0.05$; ** $P < 0.01$

ferulic acid contents are thought to greatly affect antioxidant activity. However, the study by Tachakittirungrod et al. [43] reported that the total polyphenol content had a high correlation with reducing power, but a relatively low correlation with radical scavenging activity. The results above suggest that antioxidant activity does not necessarily increase with phenol content and interaction with active substances, other than phenolic compounds, could result in slight differences in antioxidant activity. Therefore, active substances in *A. muricata*, such as acetogenin and alkaloids, should be studied in the future for the further development of enhanced functional food products.

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